EXHIBIT EE

American National Standard

ANSI/AAMI/ISO 10993-2:1993

10993-2

Biological evaluation of medical devices—Part 2: Animal welfare requirements

Approved 20 October 1993 by
Association for the Advancement of Medical Instrumentation

Approved 15 December 1993 by
American National Standards Institute, Inc.

Abstract:

This standard covers minimum requirements for the use of animals in biological testing. It is also intended to establish guidelines which allow the scientist to respect life in general, to reduce the number of animal experiments and the number of animals used in experiments, among other ways by optimization of those performed, and to minimize suffering and maintain the quality of life of the animals used in the experiments.

Contents

Committee representation

Association for the Advancement of Medical Instrumentation

The adoption of ISO 10993-2:1992 as an American National Standard was initiated by the AAMI Biological Evaluation Committee, which also functions as a U.S. Technical Advisory Group to the relevant work in the International Organization for Standardization (ISO). U.S. representatives from the AAMI Animal Welfare Working Group (U.S. Sub-TAG for ISO/TC 194/WG 3), chaired by John G. Miller of the National Institutes of Health, played an active part in developing the ISO standard.

The **AAMI Biological Evaluation Committee** has the following members:

Members:	James M. Anderson, MD, PhD, Case Western Reserve University
	Sumner A. Barenberg, PhD, Bernard Technologies
	Arthur J. Coury, PhD, Society for Biomaterials
	Roger Dabbah, PhD, U.S. Pharmacopeial Convention, Inc.
	Paul Didisheim, MD, National Heart, Lung, and Blood Institute
	Robert L. Fuson, MD, Bristol-Myers Squibb
	Donald F. Gibbons, 3M Life Sciences Sector
	John G. Miller, DVM, National Institutes of Health
	Sharon Northup, PhD, Baxter Healthcare Corporation
	Barry F. Page, Health Industry Manufacturers Association
	John Paulson, PhD, Ethicon, Inc.
	Adelbert L. Stagg, PhD, Cato Research, Ltd.
	John W. Stanford, PhD, American Dental Association
	Mel Stratmeyer, PhD, FDA Center for Devices and Radiological Health
Alternates:	Stephen Hilbert, Society for Biomaterials
	Ed Mueller, FDA Center for Devices and Radiological Health
	Harold Stanley, DDS, American Dental Association
	Wava Truscott, PhD, Baxter Healthcare Corporation

The **AAMI Animal Welfare Working Group** has the following members:

Chairman:	John G. Miller
Members:	Richard W. Bianco, University of Minnesota
	W. Jean Dodds, DVM, New York State Department of Health
	Michael J. Kallok, PhD, FACC, Medtronic, Inc.
	John G. Miller, DVM, National Institutes of Health
	David H. Mueller, Medtronic, Inc.
	Harold Stanley, DDS, American Dental Association
	Mel Stratmeyer, PhD, FDA Center for Devices and Radiological Health
	John E. Willson, DVM, Johnson & Johnson
	Andreas F. von Recum, PhD, Clemson University

NOTE—Participation by federal agency representatives in the development of this standard does not constitute endorsement by the federal government or any of its agencies.

Background of ANSI/AAMI adoption of ISO 10993-2:1992

Animal welfare requirements

As indicated in the foreword to the main body of this document (page vi), the International Organization for Standardization (ISO) is a worldwide federation of national standards bodies. The United States is one of the ISO members that took an active role in the development of the first edition of the standard for animal welfare requirements.

AAMI and ANSI procedures require that standards be reviewed and, if necessary, revised every five years to reflect technological advances that may have occurred since publication. AAMI also encourages its committees to harmonize their work with international standards as much as possible.

The 10993 series of standards was created by Technical Committee ISO/TC 194, Biological evaluation of medical devices, to fill a need for the international harmonization of test methods for various kinds of biological aspects of medical devices.

This standard was developed in response to a dramatic increase in collaborative research involving foreign countries, with widely varying animal welfare requirements.

U.S. participation in this ISO activity is through the U.S. Technical Advisory Group for ISO/TC 194, administered by the Association for the Advancement of Medical Instrumentation. The United States had a considerable contribution to this standard in their moderation of "severe" language to find common denominators that enabled broad applicability and ensured congruence of the International Standard and existing U.S. requirements.

The AAMI Biological Evaluation Committee (U.S. Technical Advisory Group for ISO/TC 194) supports international harmonization of methods used in evaluating biocompatibility of medical devices in order to help reduce unnecessary repetition of testing. The committee recommended in 1992 that AAMI initiate adoption of ISO 10993-2 in the United States as a new American National Standard and the proposal was approved 15 December 1993.

The concepts incorporated in this standard should not be considered inflexible or static. This standard, like any other, must be reviewed and updated periodically to assimilate progressive technological developments. To remain relevant, it must be modified as technological advances are made and as new data come to light.

Suggestions for improving this standard are invited. Comments and suggested revisions should be sent to Standards Department, AAMI, 3330 Washington Boulevard, Suite 400, Arlington, VA 22201.

NOTE—Beginning with the ISO foreword on page vi, this American National Standard is identical to ISO 10993-2:1992 with the exception of the explanatory note to clause 3.5.

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75% the member bodies casting a vote.

International Standard ISO 10993-2 was prepared by Technical Committee ISO/TC 194, Biological evaluation of medical devices.

ISO 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

— *Part 1:* *Guidance on selection of tests*

— *Part 2:* *Animal welfare requirements*

— *Part 3:* *Tests for genotoxicity, carcinogenicity and reproductive toxicity*

— *Part 4:* *Selection of tests for interactions with blood*

— *Part 5:* *Tests for cytotoxicity:* in vitro *methods*

— *Part 6:* *Tests for local effects after implantation*

— *Part 7:* *Ethylene oxide sterilization residuals*

— *Part 8:* *Clinical investigation*

— *Part 9:* *Degradation of materials related to biological testing*

— *Part 10:* *Tests for irritation and sensitization*

— *Part 11:* *Tests for systemic toxicity*

— *Part 12:* *Sample preparation and reference materials*

Future parts will deal with other relevant aspects of biological testing.

Annex A of this part of ISO 10993 is for information only.

Introduction

The protection of humans is the primary goal of the ISO 10993 series of standards. A second equally important goal is to ensure welfare and to minimize the number and exposure of the laboratory animals.

This part of ISO 10993 was developed to ensure the welfare of animals used in biological evaluation testing. Therefore, minimum requirements for the care and use of animals are stated.

A list of international documents concerning the care and handling of animals in biomedical research is given in annex A for information.

Biological evaluation of medical devices—Part 2: Animal welfare requirements

1 Scope

This part of ISO 10993 specifies minimum requirements for the use of animals in biological testing.

This part of ISO 10993 is also intended

a) to establish guidelines which allow the scientist to respect life in general;

b) to reduce the number of animal experiments and the number of animals used in experiments, among other ways by optimization of those performed;

c) to minimize suffering and maintain the quality of life of the animals used in the experiments.

This part of ISO 10993 applies to the experimentation performed on vertebrates. It does not apply to experimentation performed on less differentiated animals; nor does it apply to that part of the experimental work performed on isolated tissues and organs.

This part of ISO 10993 also makes recommendations concerned with the aim of reducing the number of animals used for biocompatibility testing and when possible abolishing animal experiments in this area.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 10993. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10993-1:1992, *Biological evaluation of medical devices—Part 1: Guidance on selection of tests.*

3 Definitions

For the purposes of this part of ISO 10993, the definitions given in ISO 10993-1 and the following definitions apply.

3.1 animal: Any live non-human vertebrate, excluding fetal or embryonic forms, unless otherwise qualified.

3.2 experimental animal: Animal used or to be used in experiments.

3.3 bred animal: Animal specially bred for use in experiments in facilities accredited by, or registered with, the competent authority.

3.4 animal experiment: Any use of an animal for scientific purposes which may cause it pain, anxiety, suffering, distress or lasting harm, excluding the least painful methods accepted in modern veterinary or laboratory practice (i.e., "humane" methods) of killing or marking an animal.

An experiment starts when an animal is first prepared for use and ends when no further observations are to be made for that experiment.

NOTE 1—The prevention, elimination and minimization of pain, suffering, distress or lasting harm by the successful use of anesthesia or analgesia or other methods does not place the use of an animal outside the scope of this definition.

3.5 competent authority: That authority designated by each state[1] as being responsible for supervising the experiments within the scope of this part of ISO 10993.

3.6 properly anesthetized: Deprived of sensation by methods of anesthesia (whether local or general) as effective as those used in good veterinary practice.

3.7 humane method of killing: Killing of an animal with a minimum of physical and mental suffering.

NOTE 2—Appropriate means will vary according to the animal species.

3.8 unnecessary repetition: Duplication of the same experiment without scientific need.

NOTE 3—If experimental results are properly confirmed, further repetitions are considered unnecessary. This statement does not apply to the necessary controls within an experiment.

[1] Explanatory note (American National Standard version only)—In the United States, animal welfare is regulated at the federal level, therefore "state" should be substituted with the word "country."

4 Requirements

NOTE 4—See annex A for bibliographical references.

4.1 Sequence of *in vitro* and *in vivo* tests

Animal experiments shall not be performed before appropriate *in vitro* tests, if available, have been carried out.

If the *in vitro* tests clearly show that the material, device or extract is unsuitable the animal experiment shall not be performed.

4.2 Prevention of unnecessary repetition

Scientists proposing to conduct biological evaluation tests shall make diligent efforts to ascertain that any proposed animal experiments have not been done previously. Scientists conducting biological evaluation tests are encouraged to publish the results of their experiments including negative ones in internationally referenced journals, using key words that allow identification or relevant animal experiments.

Licensing authorities are to be encouraged to establish specific lines of communication directed toward preventing unnecessary repetition. (See 5.2).

4.3 Availability of results

It is strongly recommended that the results of appropriately performed and evaluated tests be accepted by all countries.

4.4 Qualification of persons involved

All persons involved in performing animal experiments shall be

a) appropriately qualified;[1)]

b) suitably trained in the humane care of the animal species being used;

c) trained in all appropriate legislation;

d) trained in the scientific aspects of the research being conducted.

4.5 Care and handling

Care and handling of the animals shall conform to accepted animal husbandry guidelines. Care and handling of the animals shall prevent distress and pain as far as possible. See annex A.

4.6 Surgical procedure

All surgical procedures on experimental animals, especially those from which the animals are allowed to recover, shall be performed on properly anesthetized animals using appropriate aseptic procedures and careful handling of tissues involved.

4.7 Pre-, per- and post-operative care

All surgical procedures on experimental animals from which the animals are allowed to recover shall include appropriate provisions for pre-, per- and post-operative care of the animals in accordance with established veterinary medical and nursing practices.

If pre-, per- and post-operative pain is discerned, it shall be recorded and, unless precluded for scientific reasons, it shall be alleviated through the use of appropriate methods of analgesia or the experiment shall be terminated.

4.8 Planning of experiment

The design of the experiment should be appropriate to meet the desired objectives.

The design of an animal experiment shall be specified in an Experimental Plan. In addition, the investigator shall consider the use of non-invasive or alternative methods of investigation to reduce the number of animals used in the experiment (see 4.9).

The Experimental Plan shall contain the following, as appropriate:

a) details of the statistical methods to be applied before and, if necessary, throughout the entire experiment starting with the design of the Experimental Plan and ending with the Final Report;

b) essential information about the composition of the device or material and about the use of the device or material under investigation;

c) the specific goals and the scientific questions to be investigated in the study;

d) the procedures used to conduct the experiment (which should be appropriate to the device or material under investigation) including:

1) the species and approximate number of animals to be used,

2) the rationale for involving animals, and for the appropriateness of the species and numbers used,

3) the origin of the animal in order to minimize the use of animals not bred for experimentation,

4) a description of the proposed use of the animals,

5) a description of any method of euthanasia to be used.

All control procedures and comparators, whether real, standardized or simulated, shall be specified.

4.9 Reduction of animal experiments

The final intention of ISO 10993 is to forego the need for animal experiments. Toward that goal, the planning of the experiment shall consider the use of the least invasive test methods in an animal and/or the reduction of animal experiments by using less invasive methods in the same animal.

4.10 Evaluation

The evaluation of test results shall be thorough and statistical evaluation shall be performed when required.

[1)] The personality and attitude play an important role in this respect.

4.11 Multiple experiments in same animal

In general an animal shall not be used for more than one experiment in a series. The need to avoid undue suffering in the animals used should take precedence over the need to reduce the number of animals used.

4.12 Methods of euthanasia

Methods of euthanasia employed at the termination of animal experiments shall produce rapid unconsciousness and subsequent death without evidence of pain or distress.

5 Recommendations

Recommendations concerned with the future scientific study to reduce the number of animals used in biological testing, to refine the experimental methods to reduce or eliminate pain in animals, and to replace animal experiments by other means, are given in 5.1 to 5.6.

5.1 Alternative methods

Priority should be given by competent authorities, funding agencies and scientists to the validation and/or development of alternative methods. One way in which this could be achieved is to encourage editors of scientific journals to publish papers which describe alternative methods and negative results.

5.2 Database for prevention of unnecessary repetition

International databases should be established to minimize unnecessary repetition.[2)]

5.3 Animal care and handling—International documents

It is strongly recommended that internationally accepted detailed documents be produced and updated regarding the care and handling of experimental animals.

5.4 Reduction in animal usage

It is strongly recommended that authorities require only the minimum possible number of animal experiments to be performed in order to yield meaningful data and not maximum precision.

5.5 Pilot experiments

Pilot experiments should be performed to allow planning of a minimum number of experiments to provide the required result. If in a standard test the minimum number of animals required is given, that number takes precedence.

5.6 Guidelines for animal husbandry

It is requested that documents on updated animal husbandry guidelines be forwarded to ISO/TC 194.

[2)] While appreciating the question of confidentiality, it is recommended that this should not preclude the creation of the database.

Annex A
(informative)

Bibliography

[1] Directive 86/609/EEC, *Council Animal Protection Directive*. 1986-11-24.

[2] CIOMS. *International Guiding Principles for Biomedical Research Involving Animals.*

[3] Decision of the Council concerning the mutual acceptance of data in the assessment of chemicals OECD C (81) 30 (final) (1981-05-12).

[4] EN 45001:1990, *General criteria for the operation of testing laboratories.*

[5] Animal Welfare Act of 1968 (PL 89-544) as amended by the Animal Welfare Act of 1970 (PL 74-579), the Animal Welfare Act of 1985 (PL 99-198).

[6] *The Guide for the Care and Use of Laboratory Animals*, NNI publication No. 85-23 (revised 1985).

[7] Australian *Code of Practice for the Care and Use of Animals for Scientific Purposes.*

[8] Home Office Animals (Scientific procedures) Act 1986 (London). *Code of Practice for the housing and care of animals used in scientific procedures.*

[9] German *Animal welfare act*, 1986-08-18 (BG Bl.I 320).

[10] Law concerning the protection and control of animals (Japan).

NOTE 5—Other documents, in accordance with 5.6, will be added when available.